chemotaxis, chemotaxis of human thymocytes to TECK and SDF1α, both of which mediate thymocyte chemotaxis (data not shown) was examined. Anti-GPR-9-6 mAb 3C3 blocked thymocyte and CD4 lymphocyte chemotaxis to TECK. The anti-GPR-9-6 mAb 3C3 had no effect on TARC-induced chemotaxis of CD4 lymphocytes, indicating that the effect is specific (Figures 11A-11C). These results indicate that GPR-9-6 is the major physiological receptor for TECK.

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Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i-v).

REMARKS

This Supplemental Amendment conforms the specification to the Formal Drawings that were filed in the U.S. Patent and Trademark Office concurrently with Amendment A on April 4, 2003. In the Formal Drawings, Figures 3, 6, 7 and 10 as originally filed are re-labeled as FIGS. 3A-3I, 6A-6F, 7A-7F and 10A-10F, respectively. In addition, solid features of these drawings are now stippled. Accordingly, the specification has been amended as follows:

The paragraph at page 8, lines 9 through 13 has been amended to recite "stippled profile."

The paragraph at page 8, lines 14 through 22 has been amended to refer to Figures 3A-3I, and to recite "stippled profiles."

The paragraph at page 8, line 23 through page 9, line 5 has been amended to recite "stippled profile" and "stippled profiles."

The paragraph at page 9, lines 16 through 24 has been amended to refer to Figures 6A-6F.

The paragraph at page 9, line 25 through page 10, line 6 has been amended to refer to Figures 7A-7F.

The paragraph at page 10, line 24 through page 11, line 5 has been amended to refer to Figures 10A-10F.

The paragraph bridging pages 64 and 65 has been amended to refer to "Figures 3A-3C" and "Figures 3D-3I."

The paragraph bridging pages 65 and 66 has been amended to refer to "Figures 6A-6F."

The paragraph at page 66, lines 3 through 7 has been amended to refer to "Figures 7A-7F."

The paragraph at page 68, lines 1 through 10 has been amended to refer to "Figures 10A-10F."

The amendments to the specification are supported by the application as filed. Therefore, this amendment adds no new matter.

Information Disclosure Statement

A Supplemental Information Disclosure Statement (SIDS) was filed on April 4, 2003. Acknowledgment of consideration of the information provided therein is requested in the next Office Communication.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

The paragraph at page 8, lines 9 through 13 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Figures 2A-2B illustrate the specific binding of mAb 3C3 to GPR-9-6 transfectants. In Figure 2A, GPR-9-6/L1.2 transfectants were stained with mAb 3C3 ([~~solid~~] stippled profile), anti-CCR6 antibody (··) or with a murine IgG2b mAb (----) (n=2). In Figure 2B, CCR6/L1.2 transfectants were stained with mAb 3C3 (··), anti-CCR6 antibody ([~~solid~~] stippled profile) or with a murine IgG2b mAb (----) (n=2).

The paragraph at page 8, lines 14 through 22 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

[~~Figure 3 is~~] Figures 3A-3I are a series of fluorescence plots which illustrate that GPR-9-6 is expressed on B lymphocytes and subsets of CD4 and CD8 lymphocytes. mAb 3C3 was used in two color studies on mononuclear cells along with anti-CD4 FITC (Figure 3A), anti-CD8 FITC (Figure 3B), anti-CD19 FITC (Figure 3C), anti-CD56 Cychrome (Figure 3D) and anti-CCR3 FITC (Figure 3E) [~~and anti-CD56 Cychrome~~]. For thymocytes (Figure 3F), two color studies were performed with mAb 3C3 and anti-TcR Cychrome. GPR-9-6 expression on monocytes (Figure 3G), eosinophils (Figure 3H) and neutrophils (Figure 3I) was evaluated in one color studies using isolated populations of these cells and mAb 3C3 (—) and IgG2b controls (----). Anti-CCR2, anti-CCR3 and anti-CXCR2 antibodies were used as positive controls for monocytes, eosinophils and neutrophils, respectively ([~~solid~~] stippled profiles) (n=3).

The paragraph at page 8, line 23 through page 9, line 5 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Figures 4A-4H are plots illustrating that GPR-9-6 is not expressed on immature dendritic cells (IMDC), mature dendritic cells (MDC) or T_H1/T_H2 lymphocytes. Mature (—) and immature dendritic cells ([~~solid~~] stippled profile) were stained with anti-CCR5 (Figure 4A), anti-CD83 (Figure 4B), anti-CD86 (Figure 4C) or anti-GPR-9-6 (Figure 4D). Staining with IgG2b control on IMDCs ( ) is also shown. Figure 4E shows staining of umbilical CD4 lymphocytes with anti-CXCR4 ([~~solid~~] stippled profile), anti-GPR-9-6 (—) and IgG2b ( ). Figures 4F-4H show staining of T_H1 ([~~solid~~] stippled profiles) and T_H2 (—) lymphocytes with anti-CXCR3 (Figure 4F), anti-$\alpha4\beta7$ (Act1) (Figure 4G) or anti-GPR-9-6 (mAb 3C3) (Figure 4H) as indicated, with ( ) representing staining with an IgG2b control on T_H1 lymphocytes (n=3).

The paragraph at page 9, lines 16 through 24 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

[~~Figure 6 is~~] Figures 6A-6F are a series of fluorescence plots illustrating that GPR-9-6 is expressed on $\alpha4\beta7^{high}$ $CLA^{+/-}$ $CD4^+$ memory lymphocytes. Mononuclear cells were stained in three color experiments using anti-CD4 cychrome to gate on CD4 lymphocytes. The cells were also stained with anti-GPR-9-6 mAb 3C3 followed by $F(ab')_2$ anti-mouse IgG phycoerythrin to study GPR-9-6 expression on subsets defined with anti-αE (HML1, Beckman Coulter, Inc., Fullerton, CA) (Figure 6A), anti-β7 (Fib504, PharMingen, San Diego, CA) (Figure 6B), anti-CD49d (HP2/1, PharMingen, San Diego, CA) (Figure 6C), anti-CLA (HECA 452, PharMingen, San Diego, CA) (Figure 6D), anti-CD45RO (UCLH1, PharMingen, San Diego, CA) (Figure 6E) and anti-CD62L (CD56)(PharMingen, San Diego, CA) (Figure 6F) (n=5).

The paragraph at page 9, line 25 through page 10, line 6 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

[~~Figure 7 is~~] Figures 7A-7F are a series of fluorescence plots illustrating the expression of GPR-9-6 on CD4 lymphocytes in relation to other chemokine receptors. Mononuclear cells were stained in three-color experiments using anti-CD4 cychrome to gate on CD4 lymphocytes. The cells were also stained with anti-GPR-9-6 mAb 3C3 followed by $F(ab')_2$ anti-mouse IgG coupled to

phycoerythrin to study GPR-9-6 expression on subsets defined with anti-CCR2 (R&D Systems, Minneapolis, MN) (Figure 7A), anti-CCR5 (PharMingen, San Diego, CA) (Figure 7B), anti-CCR6 (R&D Systems, Minneapolis, MN) (Figure 7C), anti-CXCR3 (1C6, Leukosite, Inc., Cambridge, MA (now Millennium Pharmaceuticals, Cambridge, MA)) (Figure 7D), anti-CXCR4 (PharMingen, San Diego, CA) (Figure 7E) and anti-CXCR5 (R&D Systems, Minneapolis, MN) (Figure 7F), all of which were coupled to phycoerythrin (n=2).

The paragraph at page 10, line 24 through page 11, line 5 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

[~~Figure 10 is~~] Figures 10A-10F are a series of histograms illustrating that a subset of CD4 lymphocytes and thymocytes chemotax to TECK. $CD4^+$ lymphocytes (Figure 10F), $CD8^+$ lymphocytes (Figure 10B), $CD56^+$ NK cells (Figure 10D) and $CD14^+$ monocytes (Figure 10A) were isolated from mononuclear cells using the appropriate Miltenyi Beads. Neutrophils (Figure 10E) were isolated by dextran precipitation followed by Ficoll and eosinophils (Figure 10C) separated from neutrophils by depletion with anti-CD16 Miltenyi Beads. Uncoated 3 μm Costar plates were used to assess chemotaxis with these leukocyte subsets, with the exception of eosinophils and neutrophils, for which ECV304 monolayers were grown over the inserts before the assay. In each case, TECK was tested in a dose response fashion between 1 nM and 220 nM. Chemokines known to act on the leukocyte subsets were used as positive controls (n=2).

The paragraph bridging pages 64 and 65 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

In initial two color studies of peripheral blood, GPR-9-6 was found to be expressed on a small subset (2-4%) of CD4 lymphocytes as well as on a very small subset of CD8 lymphocytes [~~(Figure 3)~~], while B lymphocytes expressed low and heterogeneous levels of GPR-9-6 (Figures 3A-3C). Monocytes, basophils, eosinophils, neutrophils and NK cells did not express GPR-9-6 under the conditions used ([~~Figure 3~~] Figures 3D-3I). GPR-9-6 was expressed on a large subset of thymocytes expressing all levels of TcR, although a small subset of $TcR^{high}GPR\text{-}9\text{-}6^{-ve}$ thymocytes

was evident. In three-color experiments, GPR-9-6 was found on the majority of CD4, CD8 and $CD4^{+ve}CD8^{+ve}$ thymocytes and on approximately 50% of immature $CD4^{-ve}CD8^{-ve}$ thymocytes (data not shown). No expression of GPR-9-6 was seen on either immature or mature dendritic cells (Figure 4D). However, as expected, immature dendritic cells expressed CCR5, which was down-regulated on LPS activation, while CD83 and CD86 were up-regulated (Figures 4A-4C). In examining a large panel of cell lines GPR-9-6 was found on several T cell lines (Table 1). Umbilical CD4+ lymphocytes did not express GPR-9-6 (Figure 4E) and chronic activation of these cells in the presence of IL-12 or IL-4 to generate T_H1 or T_H2 lymphocytes failed to induce the expression of GPR-9-6 (Figure 4H). However, as expected, CXCR3 were clearly up-regulated on T_H1 lymphocytes (Figure 4F), while $\alpha4\beta7$, an integrin utilized in lymphocyte trafficking to mucosal sites, was up-regulated on both T_H1 and T_H2 lymphocytes (Figure 4G).

The paragraph bridging pages 65 and 66 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

The small subset of CD4 lymphocytes that express GPR-9-6 were examined in more detail by three-color staining ([~~Figure 6~~] Figures 6A-6F). The CD4 lymphocytes that express [~~GPR-9-6~~] GPR-9-6 were mainly of memory phenotype, and those cells that expressed the highest levels of GPR-9-6 were all of memory phenotype. Interestingly, memory CLA^{+ve} CD4 lymphocytes, which traffic to skin, did not express GPR-9-6. In contrast, a subset of memory $\alpha4\beta7^{high}$ CD4 lymphocytes, which traffic to mucosal sites, clearly expressed GPR-9-6. The subset of memory CD4 lymphocytes defined by expression of $\alpha E\beta7$ were also clearly subdivided into GPR-9-6 positive and negative subsets. GPR-9-6^{high} CD4 lymphocytes did not express CD62L, a homing receptor which is involved in trafficking to peripheral lymph nodes, while a small subset of GPR-9-$6^{dull}CD62L^{+ve}$ lymphocytes was evident.

The paragraph at page 66, lines 3 through 7 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

GPR-9-6^{+ve} CD4 lymphocytes were also examined for co-expression of other chemokine receptors known to be expressed on CD4 lymphocytes ([~~Figure 7~~] Figures 7A-7F). While GPR-9-6 was clearly found on both positive and negative subsets of CCR5, CCR6, CXCR3 and CXCR5, CD4 lymphocyte expression of CCR2 and GPR-9-6 was mutually exclusive.

The paragraph at page 68, lines 1 through 10 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Leukocyte subsets were also tested ([~~Figure 10~~] Figures 10A-10F) to determine if they chemotaxed to TECK. As observed in the mouse, neutrophils, monocytes, eosinophils, CD8 and NK cells did not chemotax to TECK, but did chemotax to other chemokines. However, TECK was chemotactic for a minor subset of CD4 lymphocytes. As murine TECK induces thymocyte chemotaxis, chemotaxis of human thymocytes to TECK and SDF1α, both of which mediate thymocyte chemotaxis (data not shown) was examined. Anti-GPR-9-6 mAb 3C3 blocked thymocyte and CD4 lymphocyte chemotaxis to TECK. The anti-GPR-9-6 mAb 3C3 had no effect on TARC-induced chemotaxis of CD4 lymphocytes, indicating that the effect is specific (Figures 11A-11C). These results indicate that GPR-9-6 is the major physiological receptor for TECK.